Butterflies in bags: permanent storage of Lepidoptera in glassine envelopes

Max Caspers[1], Luc Willemse[1], Eulàlia Gassó Miracle[1], Erik J. van Nieukerken[1]

1 *Naturalis Biodiversity Center, PO Box 9517, NL-2300 RA Leiden, The Netherlands; max.caspers@naturalis.nl*

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Abstract. In terms of amateurs and professionals studying and collecting insects, Lepidoptera represent one of the most popular groups. It is this popularity, in combination with wings being routinely spread during mounting, which results in Lepidoptera often taking up the largest number of drawers and space in entomological collections. As resources grow increasingly scarce in natural history museums, any process that results in more efficient use of resources is a welcome addition to collection management practices. Therefore, we propose an alternative method to process papered Lepidoptera: a workflow to digitize (imaging and data registration) papered specimens and to store them (semi)permanently, still unmounted, in glassine envelopes. The mounting of specimens will be limited to those for which it is considered essential. The entire workflow of digitization and repacking can be carried out by non-expert volunteers. By releasing data and images on the internet, taxonomic experts worldwide can assist with identifications. This method was tested for Papilionidae. Results suggest that the workflow and permanent storage in glassine envelopes described here can be applied to most groups of Lepidoptera.

Introduction

Butterflies and moths are amongst the organisms that have always been very popular with collectors and scientists. They also are among the most difficult invertebrates to prepare: it takes skill, time and proper tools to expertly set specimens. Hence, most collectors who are away from home do not prepare specimens straight away but store them temporarily, either pinned but not spread, or stored flat in boxes or opaque paper envelopes. Papering traditionally consists of placing specimens with the wings folded upwards in triangles of paper, or in small rectangular envelopes of glassine paper (Gibb 2015).

Over time, most natural history museums have amassed large collections of papered specimens, originating from private collectors and expeditions of these museums, and most of these have never been properly prepared. Due to lack of resources, many of these collections have been left undisturbed, often for decades, and curators are struggling how to make this material accessible for research.

In 2015 the number of papered Lepidoptera in the collection of Naturalis Biodiversity Center, Leiden, the Netherlands was estimated to consist of roughly 500,000 specimens, some being as old as 80 years. A substantial part of the backlog (>200,000 specimens) consisted of Lepidoptera collected by J.M.A. van Groenendael, a Dutch physician working in the former Dutch East Indies between 1931 and 1954 (de Boer 1998). Most butterflies and moths in the papered collection are placed in opaque envelopes (Fig. 1), which makes it impossible to check the contents without opening the

Figure 1. Overview of the contents of a drawer with papered Papilionidae*.

envelopes and risking damage. The papered Lepidoptera specimens have been partly sorted to some extent in the past. Still, the geographic and taxonomic information available on storage unit level (e.g. box or drawer), if present, is often no more specific than country and family. The individual envelopes, on the contrary, often contain more specific information on the collecting event (Fig. 2).

Though specimens in papered envelopes potentially contain a wealth of information for research, their current storage method seriously hinders study. In practice this means that such collections have been neglected for decades, with only an occasional search for interesting specimens. This involves a high risk for damage and sometimes results in damaged legs, antennae or even wings. Processing this number of Lepidoptera following current practice, namely pinning and spreading the specimens, would take up roughly 7500 drawers, not to mention the personnel it takes. As space, time and money are resources that have been growing increasingly scarce for natural history museums in the past decade (Dalton 2003; Pyke and Ehrlich 2010; Bradley et al. 2014; McLean et al. 2016), an alternative for making papered Lepidoptera collections accessible would be a welcome addition to current collection management practices.

For collections of Odonata (dragonflies and damselflies), rectangular 3×5 inch index cards and transparent envelopes of film were proposed some decades ago as a permanent storage solution for unmounted specimens (Beatty and Beatty1963). This method was to replace storing specimens in envelopes of cellophane and triangular folds of newspapers. This system has become common practice for the Odonata collections at Naturalis Biodiversity Center, using glassine envelopes (van Tol 2001). Glassine envelopes are semi-transparent, acid-free, do not build up static charge, are

* Photographs were taken by the first author unless mentioned otherwise.

Figure 2. An old drawer filled with papered Lepidoptera. The drawer contains rough information (on stickers) about locality and collector, while the individual envelopes have more specific information on locality and date written on them. Photo by Luisa de Bruin.

inexpensive and easy to use. They have been widely applied for temporary storage of entomological specimens for about forty years now (Gray 1971; Gibb 2015; Winter 2000). At Naturalis they are also used to store leaves with insect damage, such as leafmines. The proven benefit of glassine envelopes leads to the assumption they could be appropriate for Lepidoptera as well, and that the use of cards in combination with glassine envelopes could be an incredibly convenient and space efficient alternative to conventional spreading and mounting.

In order to properly process the collection of 500,000 papered butterflies and moths at Naturalis, repacking should be combined with a digitization workflow. Additionally, to potentially reduce costs, non-expert volunteers should be able to carry out this workflow. A pilot project was carried out in 2016 to put this to the test, funded by the Van Groenendael-Krijger Foundation. During this project a workflow was developed to repack and digitize large collections of unmounted Lepidoptera. This is an approach that differs from the traditional practice of mounting Lepidoptera in a way that is less time, space and money consuming, while ensuring optimal accessibility to the collection, both physical and digital. In this paper a workflow for digitizing and permanently storing unmounted Lepidoptera is presented, that resulted from the 2016 pilot. The digitization and repacking of all papered Lepidoptera is currently (June 2018) still going on. It is estimated that another ten years are required to completely deal with the backlog of papered specimens. We will continuously seek to refine the workflow and storage method, and are happy to confer with prospective users.

Why mount butterflies and moths?

Throughout the 18th and 19th centuries, collectors devised different methods to preserve delicate butterflies and moths. The apothecary James Petiver (1663–1718), for example, preserved his butterflies dried, pressed and glued in books “after the same manner you do the Plants” (Petiver [1709?]),

but he also advised to push a pin through the thorax and pin them to one's hat, until the specimen could be placed into a box (Salmon 2001). The physician and botanist Sir Hans Sloane (1660–1773) also preserved butterflies pressed on paper sheets, sometimes in combination with plants, and sometimes he placed specimens between thin layers of mica, sealed with a 'passe-partout' (MacGregor 1994). Another example of this kind of storage can be found in the beautiful collection of butterflies of the Dutch bookseller Jacob Ehrlich (1787–1863). The specimens are preserved between glass plates and placed in cases of Mahogany wood made to resemble books (Zwakhals et al. 2015). Although Petiver already mentioned the use of pins in the early 1700s, this method seems to have become more widespread only after the mid-18th century. In 1753, David Hultman, in a work supervised by Carl Linnaeus, advised to spread legs, antennae and wings of insects and to preserve them dried and pinned, taking extra care not to damage the delicate butterfly scales (Hultman 1753). Linnaeus also kept his insects dried, spread and pinned (Mikkola and Honey 1993).

Regarding the methods to spread Lepidoptera, the artist Benjamin Wilkes (died c. 1749) was one of the first to write down instructions for setting butterflies and moths in his "Directions for Making a Collection" (Wilkes 1742; Salmon 2011). So, as early as the mid-18th century, many collectors preserved butterflies and moths air-dried, wings spread, and pinned in boxes or drawers lined with cork or other material. It is not only pleasant to look at, but it also facilitates the examination of the wings and body. The combination of pinning and spreading made the specimens easy to handle, it provided space for labels, and is the best way to study them from all sides. All the specimens from this earlier period, either pinned, pressed or placed between layers of glass, were spread in a 'natural' position, that is, with the leading edge of the forewings nearly perpendicular to the body and behind the head. Since the turn of the 19th century, Lepidoptera are always mounted so that the forewings are pushed forward far enough so that their hind margins form a nearly straight line and are perpendicular to the body's axis, which allows for better examination of the wings. This is ideal for detailed descriptions of external morphology and for imaging. Moreover, when the preparation of genitalia is needed, it is easy to break off the abdomen from pinned specimens without damaging the rest of the specimen. As a result, nearly all entomology and collection handbooks describe this now as the standard method of mounting butterflies and moths on boards, and describe the papering as the method for transport and temporary storage (Greene 1863; Greene and Farn 1880; Martin 1977; Schauf 2000).

More recently, new preservation and storage techniques have been developed for fresh or recently acquired material in order to facilitate morphological and molecular research, such as fluid preservation and freezing of the specimen bodies, while the wings are saved in clear plastic coin holders (see for example Cho et al. 2016 or Brower 2000). However, none of these methods have become as important as for other groups of invertebrates, so papering and pinning are still widely used to store Lepidoptera.

In order to maximize the use of storage space, curators of large collections often fill insect drawers as much as possible by letting the wings of the butterflies overlap, or in case of moths, by not spreading some of them. This has obvious disadvantages. In the case of overlapping wings, it results in overcrowded drawers, with greater risk of damage for the specimens. Unmounted moths are, on the other hand, difficult to examine. Pinning moths without spreading, that is, with their wings close to the abdomen, however, may be more efficient than papering.

For specimens belonging to easily identifiable species, often only collected to serve as a faunistic voucher, there is no direct need for mounting in order to obtain the necessary information.

This means that for long series of specimens with similar label data, the default storing method could be unmounted in envelopes in order to save space. Only when other kinds of research require a traditionally mounted specimen, it is necessary to select the specimens and spread them.

For DNA studies, papered specimens may yield even better results, as they have never been relaxed. Relaxing requires exposure to moisture and therefore may be detrimental to DNA quality (see for example Prosser et al. 2016). However, many papered collections have been in very wet climates for some time and as a consequence they may have already suffered some form of DNA degradation by either climatic conditions or the use of chemicals that prevented mould or insect damage (Zimmermann et al. 2008; Tin et al. 2014; Prosser et al. 2016).

Butterflies in bags – an innovative workflow

Here we describe a three-phase workflow developed to tackle the enormous collection of 500,000 papered Lepidoptera at Naturalis Biodiversity Center. Central to this workflow is the idea that Lepidoptera will be permanently stored in glassine envelopes, still unmounted, in contrast to mounting as the traditional method of storage. In the first phase of the workflow non-expert volunteers repack and digitize the papered Lepidoptera, while taxonomic experts take care of the identification in the second phase. The post-processing in the third phase is the responsibility of collection managers. Step by step we will treat the three sequential phases of this workflow: repacking and digitization, identification and post processing.

Material and methods

To test the designed workflow during the pilot in 2016, a selection of envelopes was made that comprised the papered Papilionidae (swallowtails) collected by J.M.A. van Groenendael in Java, Indonesia. This family of butterflies was chosen for several reasons. In the first place, it is one of the smallest families of butterflies, comprising around 180 species in the Oriental region (Parsons 1998). Secondly, because of their beauty and large size, swallowtails have been intensively collected for centuries and are well represented in museum collections. Finally, the specimens are relatively easy to identify by wing undersides, the only visible part in papered butterflies. On top of that, because of their large size, swallowtails are ideal to test the potential gain in space that can be achieved by bagging instead of mounting the specimens.

Deciding which specimens should be processed first is primarily dependent on ongoing research. In the absence of ongoing research, prioritizing the papered Lepidoptera is based on taxonomy, so the project only needs to depend on one expert at a time. Therefore, once the Papilionidae are fully processed, the rest of the papered Lepidoptera collection will be processed by family during the expected 12 year life span of the project.

To digitize and store specimen metadata for each swallowtail together with their image, the Collection Registration System (CRS) in use by Naturalis was chosen. CRS was developed during the FES Collection Digitization project (Heerlien et al. 2015) from 2010 to 2015 to store collection related data and support collection management activities. It now holds over 8 million specimen records at object level and 32 million specimens at species/storage unit level. Eventually the data and images in CRS are published on http://bioportal.naturalis.nl/, including the papered butterflies (example: http://bioportal.naturalis.nl/multimedia/ZMA.INS.1332352_1/)

Phase 1, which does not require in-depth knowledge, is carried out by volunteers in order to reduce costs. A team of volunteers was recruited sufficient to occupy three workstations five days a week. As most volunteers are available for one day per week the team includes some 20 persons to accommodate for illnesses and holidays.

The Workflow

Phase 1 – Repacking and digitization

Step 1: Pre-processing by project coordinator

1. All information available on storage unit level, such as collecting date and location or collection name, is registered in the Naturalis Collection Registration System (CRS) as first basic information for further individual registrations.
2. A stock of blank 3×5 inch index cards is printed, which contain a unique data matrix and registration number. These labels are printed on thick, 100% cotton, acid free ledger paper to ensure sustainable storage. The registration numbers are consecutive to improve usability. Labels are subsequently cut to perfectly fit the glassine envelopes. This provides support and protection for the specimen in the glassine envelope.

The result is a prepared drawer of papered Lepidoptera with a supply of unique 3×5 inch cards (Fig. 3) alongside a general supply of glassine envelopes.

Step 2: Handling by volunteers (Fig. 4)

1. The next index card as well as a random papered butterfly or moth is taken from the prepared drawer. The information written on its envelope, most commonly collecting locality and date, is registered into the CRS database.

Figure 3. A drawer with papered Lepidoptera that has been prepared by the project coordinator for the volunteers. Next to the drawer is a stock of acid free labels which will be adjoining the specimens.

Figure 4. A work station which is occupied by volunteer Herman Hillebrand. On the right is the main stock of Lepidoptera and the computer screen on the left shows the live image of the camera. Photo by Luisa de Bruin.

2. The specimen is taken out of its original envelope and is imaged together with the original envelope and the newly printed index card (Fig. 5), using a digital camera. Care is taken that the part of the original envelope containing the source data is imaged, so the quality of transcription can always be checked.
3. The label is inserted into a glassine envelope, after which the specimen carefully follows. The glassine envelopes are finally placed in numerical order by their registration code, in small cardboard boxes that fit in tailor made drawers (Fig. 6). The data matrix on the index card can be scanned through the glassine envelope. Each working station has its own series of numbers to avoid the error-prone rearranging of specimens between stations.

Step 3: Completion by the project coordinator

After volunteers have registered, imaged and stored the specimens in glassine envelopes, the project coordinator makes sure that the quality of the data is up to standard and that the digital and physical collections are properly organised. This includes a weekly quality control of the data in the generated specimen records, renaming and linking the produced images to the corresponding specimen records and ensuring the specimens and drawers are stored properly.

Figure 5. An example of the standardized photographs produced during this project. These are later sent to taxonomic experts for identification and can be used for automated image recognition. They will also be visible online on bioportal.naturalis.nl.

Figure 6. The final storage of the specimens. The specimens are visible through the semi-transparent glassine envelope, and are stored in the cardboard boxes within drawers where they are easily accessible by unique specimen number.

The entire process of phase 1 is schematically represented by a flowchart (Fig. 7). Both the description of the workflow above as the flowchart for phase 1 provide a very global overview of the sequence of actions and do not cover all exceptions to the process and specifics on movement of objects and information.

Phase 2 – identification

The images taken during step 2 of phase 1 are sent by the project coordinator to a taxonomic specialist in the respective Lepidoptera family. Using the images, the specialist identifies specimens to the lowest taxonomic level possible and registers the taxonomic information in an external file. During this process, the specialist can mark the specimen for mounting and/or DNA-sampling when deemed necessary, i.e. when identification using solely the ventral view of the wings is not possible or the specimen is very rare in collections. The project coordinator imports the specialist's identifications from the external file into the CRS database so they correspond with the specimens and accompanying records.

For the Naturalis collection of papered Lepidoptera, it is practically impossible to determine whether all specimens in a drawer selected for repacking and digitization belong to a certain family. The collection is only roughly sorted by family and the envelopes are usually opaque. Since

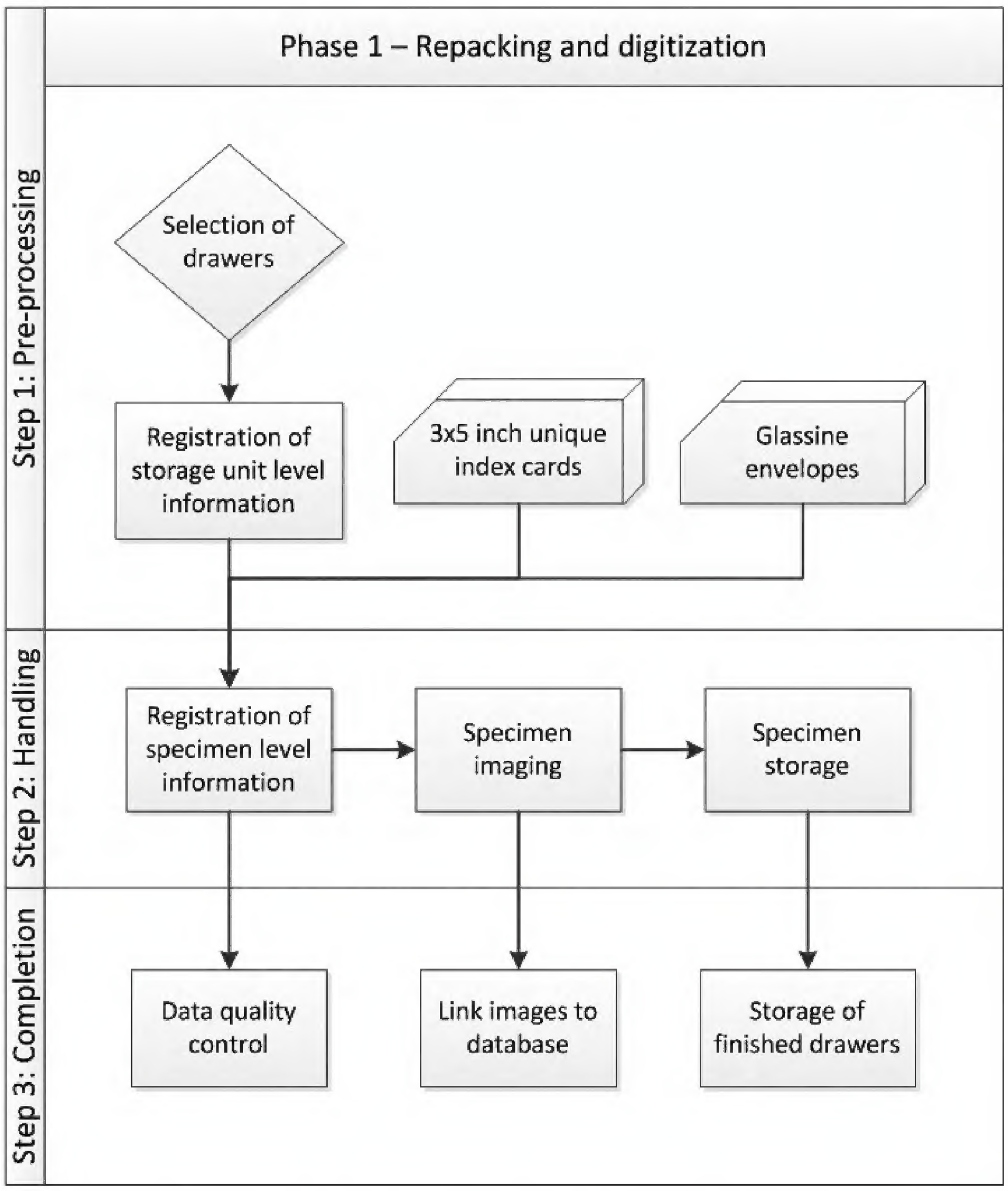

Figure 7. Flowchart of steps 1 through 3 during phase 1 of the workflow.

the volunteers are not expected to identify Lepidoptera, all specimens from prioritized drawers are processed even though they might belong to another family. In the case of the 2016 pilot, the Papilionidae expert identified the non-Papilionidae to at least family level so these can later be easily redirected to their respective expert.

At the time of writing, the project is experimenting with deep learning image recognition to reduce the time a specialist has to put in identifying specimens of common species (Hogeweg 2018; Schermer et al. 2018). This is done by using a validated set of identified and imaged collection specimens to train software, which develops a model of what the butterfly species look like. The software can then be used by volunteers to automatically identify common species, so that taxonomic specialists can focus on the rare and difficult species.

Phase 3 – post processing

The collection of Lepidoptera resulting from the first two phases, as described above, is ordered numerically by their registration code. On the other hand, the Naturalis collections of mounted Lepidoptera and papered Odonata are ordered taxonomically. A logical next step would be to reorder the butterflies in envelopes to fit this system. However, as the specimens are easily traced individually by their identification numbers, there is no direct necessity to reorder and handle all specimens again. If there is need for a taxonomically ordered collection of papered butterflies in the future, a re-curation workflow will be set up.

A more pressing issue at the moment is dealing with the specimens that are marked by the expert to be mounted or DNA sampled, for example, when identification was not possible using only the photograph. These specimens will be extracted from the papered collection and professionally mounted for further examination.

Results for curation

The described workflow yields a thoroughly curated collection, both physically and digitally. The physical collection of unmounted Lepidoptera in glassine envelopes is archived sustainably and is easily accessible due to being ordered numerically. The digital collection comprises a photograph, an identification and in most cases a collection date and locality information for each individual specimen. Notably, the new workflow is especially efficient when compared to the traditional practice of mounting. During the 2016 pilot the team of volunteers digitized a total of 16,440 specimens, mostly Papilionidae, none of which required mounting for further study. The gains in time, space and costs are discussed below. In the workflow here presented, the gains in resources are by and large dependent on the percentage that still requires mounting after digitization. Because Papilionidae in this respect are not representative for all Lepidoptera, several situations that depict varying levels of mounting requirements are included in the calculations as well.

Time efficiency

In Table 1 and Fig. 8, the time required for re-curating 16,440 specimens of Papilionidae is presented. The 'traditional practice' value is visualised as a dotted line in the graph because it is plotted only as a reference value for the new workflow. In the case of the 2016 pilot, 0% of the specimens

Table 1. Time required to handle and digitize 16,440 specimens of Papilionidae in four scenarios, compared to the traditional practice of direct mounting. Scenario 1 corresponds with the results of the 2016 pilot.

16,440 specimens of Papilionidae		Proposed method - scenario 1	Proposed method - scenario 2	Proposed method - scenario 3	Proposed method - scenario 4	Traditional practice - direct mounting
% that requires mounting		0	25	75	100	100
Handling time (days)						
a.	envelopes (40 ex./day)	411	411	411	411	
b.	mounting* (20 ex./day)	0	206	617	822	822
Total handling time (days)		411	617	1028	1233	822

*: includes mounting, labelling and digitizing, excludes relaxing time required for mounting.

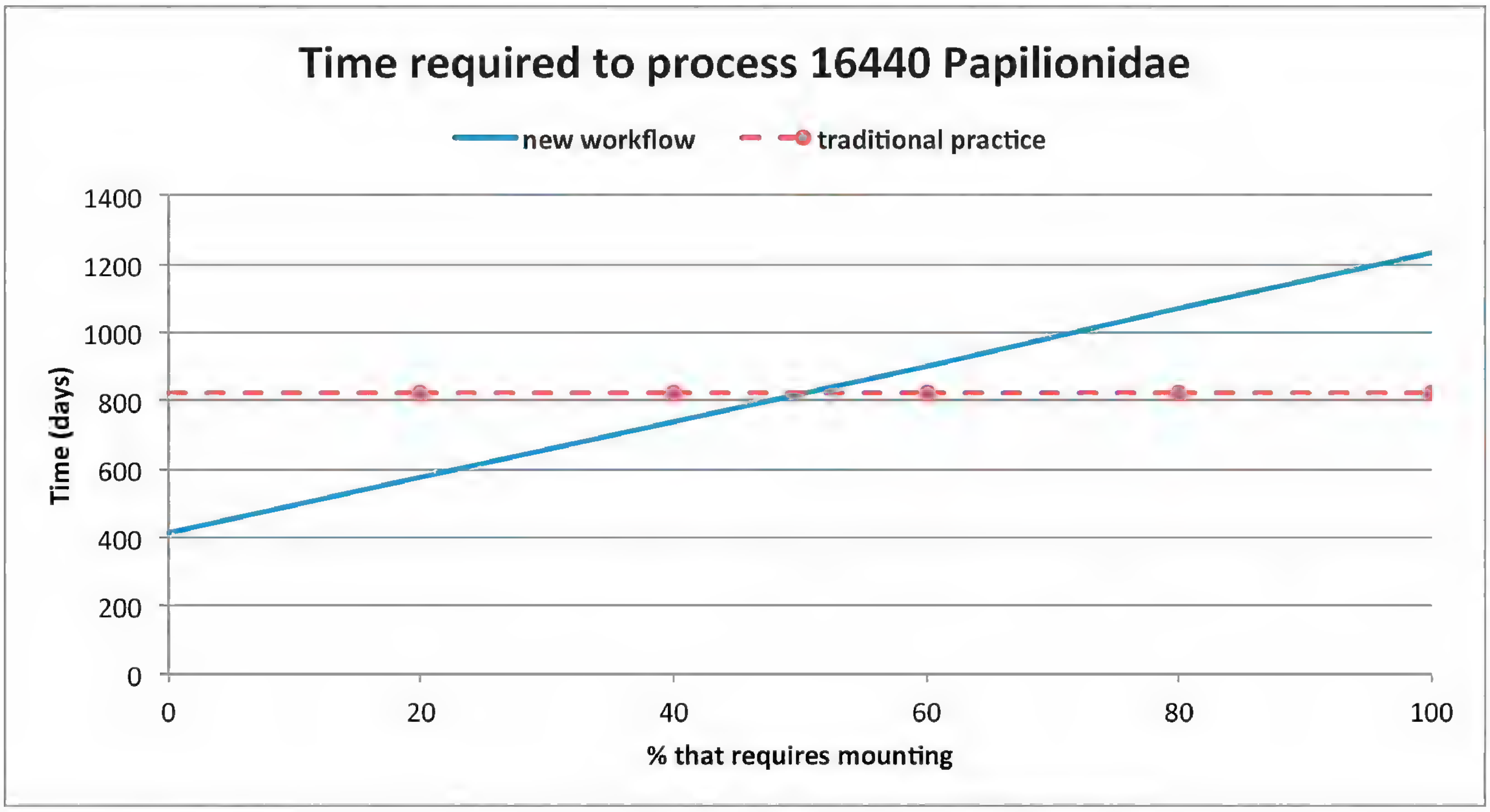

Figure 8. Time required to process 16,440 Papilionidae. When more than 50% of the digitized Lepidoptera specimens require mounting after digitization, the new workflow will become time-inefficient.

needed mounting so the new workflow required only half the time of what the traditional practice would take. The percentage of specimens that requires spreading after digitization will be handled twice. Therefore, applying the proposed method will be too time-consuming if that percentage increases past 50%.

Space efficiency

In Table 2 and Fig. 9, the number of drawers required to store 16,440 specimens of Papilionidae is presented. The 'traditional practice' value is visualised as a dotted line in the graph because it is plotted only as a reference value for the new workflow. In the case of the 2016 pilot, 0% of the specimens needed mounting so the applied method required only one seventh of the number of drawers that traditional practice would take. Even if 99% of the specimens still require mounting after digitization, storing 1% of the specimens unmounted in glassine envelopes saves space.

Table 2. Drawers required to store 16,440 specimens of Papilionidae in four scenarios, compared to the traditional practice of direct mounting. Scenario 1 corresponds with the results of the 2016 pilot.

16,440 specimens of Papilionidae	Proposed method - scenario 1	Proposed method - scenario 2	Proposed method - scenario 3	Proposed method - scenario 4	Traditional practice - direct mounting
% that requires mounting	0	25	75	100	100
Number of drawers required					
a. envelopes (350 ex./drawer)	47	36	12	0	0
b. mounting (50 ex./drawer)	0	83	247	329	329
Total number of drawers required	47	119	259	329	329

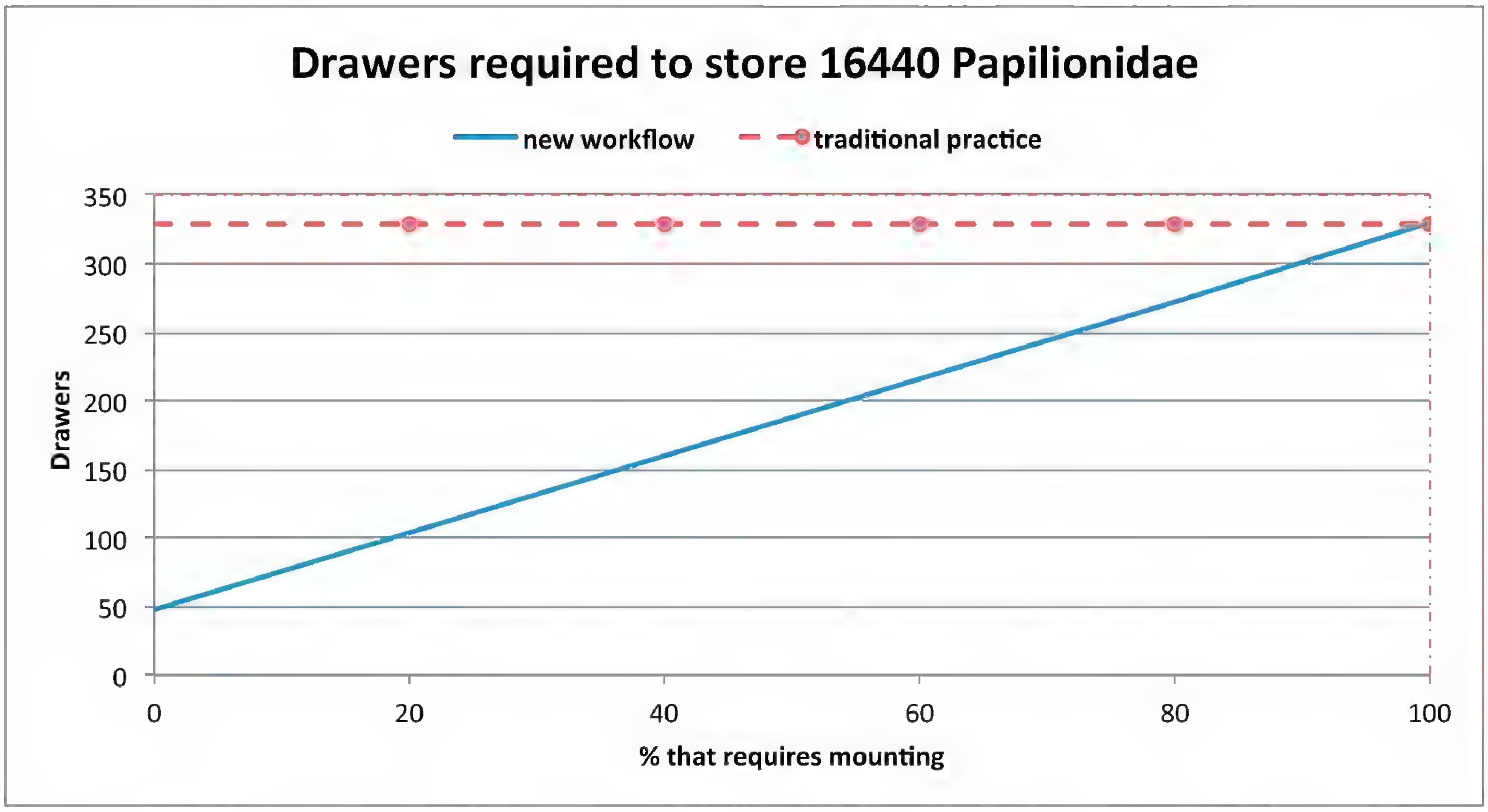

Figure 9. Drawers required to store 16,440 Papilionidae. Storing unmounted Lepidoptera specimens in glassine envelopes is always space-efficient.

Cost efficiency

In Table 3 and Fig. 10, the cost required for re-curating 16,440 unmounted specimens of Papilionidae is presented. The 'traditional practice' value is visualised as a dotted line in the graph because it is plotted only as a reference value for the new workflow. In the case of the 2016 pilot, 0% of the specimens needed mounting so the new workflow required only a third of what traditional practice would take. Even though the amount saved decreases when the percentage that still requires mounting increases, the new workflow remains profitable up until the moment that 89% of all specimens require mounting after digitization.

Discussion

A new workflow is presented for processing papered Lepidoptera specimens as an alternative to mounting all individuals. This workflow entails digitizing the specimens and repacking them, still

Table 3. Costs required to handle and digitize 16,440 specimens of Papilionidae in four scenarios, compared to the traditional practice of mounting. Scenario 1 corresponds with the results of the 2016 pilot.

16,440 specimens of Papilionidae	Proposed method - scenario 1	Proposed method - scenario 2	Proposed method - scenario 3	Proposed method - scenario 4	Traditional practice - direct mounting
% that requires mounting	0	25	75	100	100
Material costs involved* (€)	6753	10277	16376	19426	18100

*: includes drawers, cardboard boxes, glassine envelopes and acid free ledger paper. A breakdown of costs per item can be found in the online Suppl. material 1.

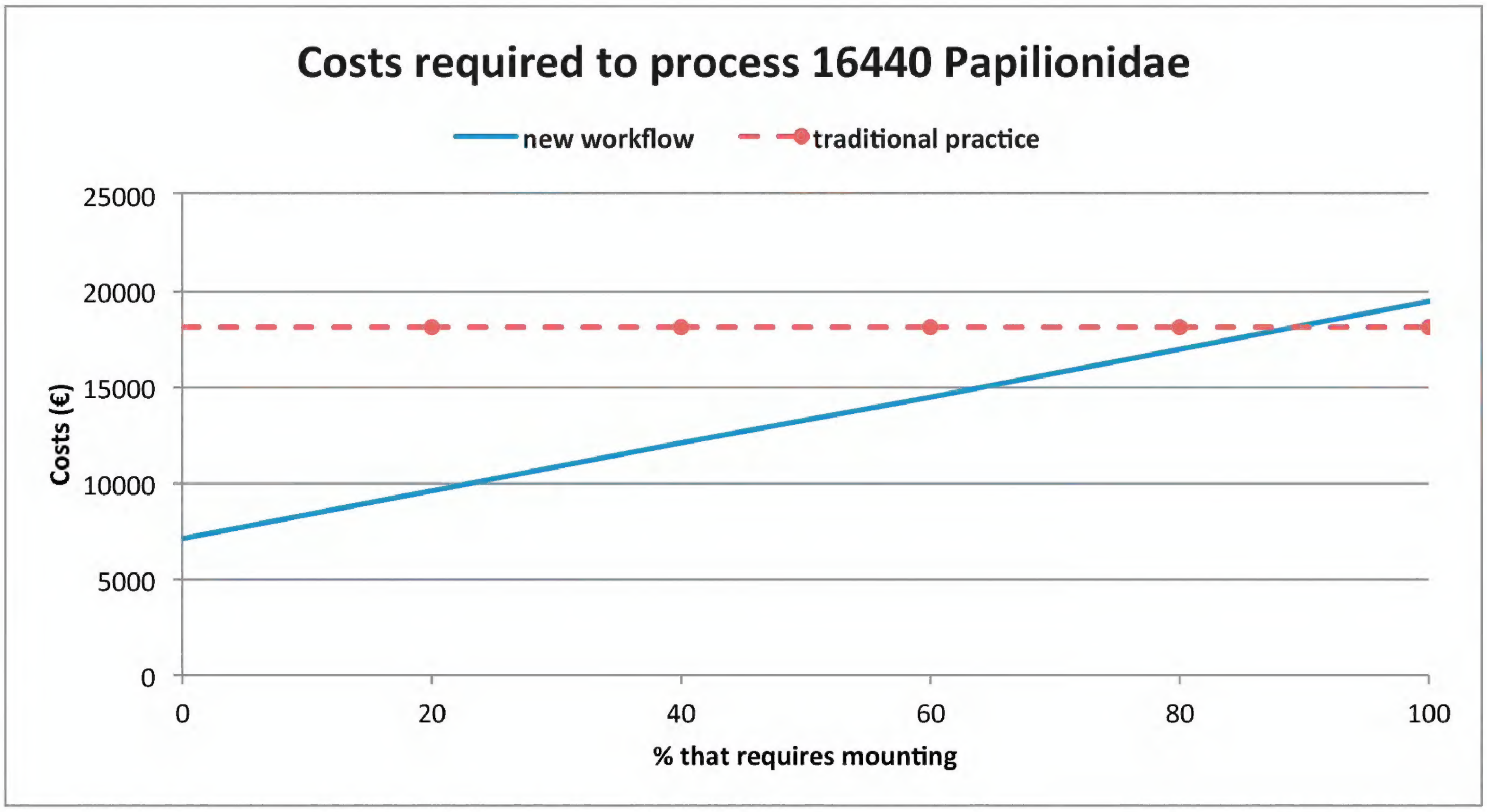

Figure 10. Cost (in euros) required to process 16,440 Papilionidae. When 89% of the papered Lepidoptera collection requires mounting after digitization, the new workflow will become cost-inefficient.

unmounted. Mounting is limited to those specimens that otherwise cannot be identified or are special or rare. Results indicate that the efficiency of this workflow depends on the number of specimens that still require mounting after processing. For Papilionidae, due to their size and relative ease of identification, saving resources when applying the new workflow is evident. For other groups, i.e. most moth families, this workflow most likely is less efficient when aiming at identifying all specimens to species level.

Nevertheless, even if identification of unmounted material is only possible to a higher taxonomic level (i.e. family or subfamily), applying this workflow is still advantageous. A large number of papered Lepidoptera will be individually processed and stored in an acid-free durable environment available for further study. Not only is a high level (family or subfamily) identification already an enormous improvement, the locality and date for each specimen becomes digitally available as well. This will facilitate research and improve selection of specimens to be mounted for further study. The photographs are disseminated online and sent to experts for identification.

When faced with the decision whether to apply this new method to process a collection of papered Lepidoptera or mount all specimens at once, being able to estimate the percentage that will require mounting is a welcome addition. So far this has proved to be quite difficult considering the historical nature of the collection with limited documentation and most envelopes being opaque. Perhaps knowledge about the collector might help in indicating what kind of Lepidoptera are to be expected, but in general the means of assessing beforehand the probable gains in time, space and cost requires further scrutiny.

Finally, future automated identification tools for unmounted Lepidoptera that recognize shape and colour patterns of the wings would perfectly fit into the workflow presented here (Schuettpelz et al. 2017; Hogeweg 2018; Schermer et al. 2018). For relatively difficult groups of Lepidoptera, this will allow for a rapid first identification and for easier groups these tools can be trained to identify specimens up to species level. We believe the workflow presented here demonstrates a promising way for processing and permanently storing unmounted Lepidoptera. It even holds the potential to be further developed and tailored to facilitate in-field registering and papering, resulting in specimens that upon arrival can be stored directly in the collection.

The original envelopes are not stored physically with the specimens, except for ones with exceptional historical information or where the source data is very hard to read and keeping the original envelope might provide beneficial. The method of digitization results in images and verbatim transcriptions of the source data on the original envelopes. This allows the source data to always be digitally accessible for inspection when there is doubt about the validity of the transcribed or interpreted data. A representative selection of the original envelopes is kept separately in our archive because of their historical value. Instead of disposing the rest of the original envelopes, alternative uses such as outreach are being explored.

Eventually a papered collection of taxonomically arranged Lepidoptera may form an important supplementary collection next to the traditional collection of mounted specimens. It is already common practice in some laboratories to keep voucher specimen used for tissue extraction for DNA in envelopes (N. Wahlberg, personal communication; Cho et al. 2016). By developing a way to store the papered Lepidoptera accessions, they can be incorporated in the main collection.

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Supplementary material 1

Permanent storage of Lepidoptera in glassine envelopes

Authors: Max Caspers, Luc Willemse, Eulàlia Gassó Miracle, Erik J. van Nieukerken

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